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13. ABSTRACT (Maximum 200 words)

Aim: development of optical techniques for diagnosis and treatment of breast cancer

Spectral analysis of white light reflected from tissue provides a rapid, non-invasive, diagnostic technique. We have collected paired optical and conventional histologic measurements from 647 sites in breast tissue and axillary lymph nodes and looked for spectral features to identify cancer. Spectral analysis techniques known as model based analysis (MBA) have been developed using artificial intelligence techniques such as neural networks and hierarchical cluster analysis coupled with innovative spectral processing that we are in the process of patenting. Our latest results show a sensitivity and specificity for detecting cancer in breast tissue or lymph nodes as given in the table.

Table: Accuracy of Optical Diagnosis of Breast Cancer

	Breast Tissue	Sentinel Nodes
Sensitivity	94%	84%
Specificity	92%	87%

Therapy aims for complete ablation of small cancers using MR guided Interstitial Laser Photocoagulation (ILP). We have shown that ILP can ablate small cancers and that contrast enhanced MR can detect untreated areas of cancer as small as 2mm. Although the number of suitable patients for study is still small an additional year of research as agreed in a revised statement of work should increase the numbers treated significantly. ILP to 58 fibroadenomas confirmed that laser necrosed tissue is resorbed and the treated area heals safely. This makes ILP a promising treatment for fibroadenomas.

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Stephen O'Brien 21st Sept 2001.

PI - Signature Date

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Interstitial Diagnosis and Treatment of Breast Tumors
Annual Report 1st September 2000 – 31st August 2001

Introduction:

This research falls broadly into two main areas:

1) *Diagnosis of malignant breast tissue by optical biopsy*: This involves placing a probe containing two optic fibers into a breast lump, onto a suspicious area of tissue during surgery or onto an excised tissue specimen such as a lymph node. One fiber is attached to a Xenon flashlamp that sends short pulses of white light into the area of tissue to be interrogated. A second optic fiber collects light that is scattered back, which is then analyzed spectroscopically. A conventional histological specimen (a biopsy) is taken from precisely the same point, and when this has been analyzed the two sets of data are compared. Algorithms for spectral analysis can subsequently be developed once sufficient data sets have been collected. In this way it is anticipated that we can train the optical system to give an accurate diagnosis of any breast lump, or predict the presence of malignant tissue in the excision margins or excised lymph nodes.

2) *Treatment of cancers and benign fibroadenomas of the breast by Interstitial Laser Photocoagulation*: The tumors to be treated are first identified by either ultrasound scanning (USS) or Magnetic Resonance Imaging (MRI). One or more small-bore cannulae are inserted into the middle of the lesion through which optic fibers are passed. These in turn are attached to a semi-conductor diode laser. The laser is activated for between 10 and 20 minutes at each fiber site. The laser causes heating of the tumor tissues and subsequent thermal necrosis. The body reabsorbs the dead tissue over a period of several months. For the fibroadenomas, follow up is with serial USS. Patients with cancer have laser treatment before routine surgery. They all have MR scans pre- and post laser treatment and just before surgery. These are subsequently compared to the histological specimen. We aim to show that fibroadenomas and small cancers can be successfully treated with laser therapy and to determine if MR imaging can detect any malignant tissue remaining after laser treatment.

In the 12 months covered by this report there have been a number of changes in personnel, including a move by one of the subcontractors and the discontinuation of the subcontract with a second. Our principle collaborator at Los Alamos, Dr Irving Bigio, has moved to Boston University as Prof. of Biomedical Engineering, but we are continuing our collaboration with him on the same terms as when he was at Los Alamos. The team led by Dr Steven Harms at Little Rock, Arkansas, have been unable to undertake the work described in their agreement with the principal investigator in London, and so with regret, that sub-contract has been terminated. A revised statement of work was submitted to the sponsors in March 2001. This was approved and the project has been extended for a year with no extra funding.

Developments in the analysis of data have led to a patent application in the field of spectral processing and for this development the U.S. Army Medical Research and Materiel Command has been informed separately. Gavin Briggs, the surgeon who had been working on the project from its inception has finished his contract and left to take up a position as a trainee radiologist in Belfast, Northern Ireland while finishing the writing of his MD thesis. Another surgeon, Andrew Lee, has joined the project and his CV is included as appendix one.

Objective 1: Advance diagnostic techniques with Optical Biopsy.

At the start of this program, little more than the basic principle of the use of elastic scattering spectroscopy for tissue diagnosis was known. In the first two years we undertook some basic experiments to understand what volume of tissue the pulse of white light was interrogating and details of these have been given in earlier reports. We have also performed experiments to determine the absorption coefficients of various commonly observed dyes and colored substances (chromophores). This has led to new ways of interpreting the spectra obtained from tissue to making the analysis simpler and as accurate as possible. We have been particularly looking at ways of eliminating spectral features due to absorption of the light by the chromophores, thereby maximizing the information available from the rest of the spectrum. These spectral processing techniques have led to a patent application and the possibility of commercializing the technology.

Experimental work:

Assessment of the volume of tissue studied

From theoretical considerations, the distance from the fiber tip from which light may contribute to the detected optical signal is about 1mm. Our experiments confirmed this result. Tissue up to a distance of 1mm from the tip of the probe could influence the spectrum, but tissue further away could not. We concluded that with the geometry of the probe as it is constructed at present the volume of tissue interrogated is of the order of $2mm^3$. Thus conventional biopsy specimens needed to be taken within about 1mm of the site of the optical measurement for accurate correlation of the two.

Absorption by hemoglobin

Usually the strongest chromophore in normal breast tissue in the visible part of the spectrum is hemoglobin (in both oxygenated and deoxygenated forms). In purely adipose tissue or if a blue dye has been used intra-operatively to locate the sentinel node then other chromophores may dominate. In our spectra, it was noticed that the hemoglobin absorption curves from different tissue types showed some obvious similarities. This raised the possibility of measuring the relative hemoglobin absorption within each spectrum. Not just might this give an approximation of the saturation (percentage oxygenation) and hematocrit levels (total hemoglobin concentration) within the tissue but also this measurement could be used to attempt to remove the hemoglobin absorption contribution from each spectrum. If this could be done, it might reveal more diagnostic information from the remaining, underlying, parts of the spectrum.

The first problem was that the absorption spectra of oxy- and de-oxy hemoglobin, although similar in overall form, have slight differences in the position, width and height of their absorption maxima. The relative amounts of oxy- and de-oxy hemoglobin vary within tissue and also the overall amount of blood in the tissue, as well as any concentration of blood between the tip of the probe and the tissue, could vary over a large range. However, the separate oxy-Hb and deoxy-Hb peaks are sufficiently different to be recognizable so two experiments were devised to quantify the values for the absorption spectra to be used on the data we collected from patients.

1) Firstly a sample of venous blood (nominal Hb 12g/dL) was acquired from one of the researchers and a series of samples with different concentrations were obtained with saline using a double dilution method. The concentrations ranged over 4 orders of magnitude i.e. 1:0 to 1:32768; blood:saline and ESS spectra were obtained from each concentration. A linear relationship was observed over 2 orders of magnitude spanning 1:256 to 1:32768 which corresponds to the concentrations observed in-vivo. Using this data we can now obtain a relative concentration of blood for any given spectra, which could then be used as an additional factor in the analysis. The same pattern was observed when the dilutant mixture contained a scatterer (Intralipid 10%).

2) To assess deoxy- and oxyhaemoglobin absorption we used a venous blood:saline concentration of 1:512 (inside the linear range) and gently bubbled pure oxygen through the solution for 5-10 minutes, until no further color change was observed in the solution. A spectrum was obtained which therefore only reflected absorption from oxy-hemoglobin, the pure oxy-Hb absorption spectrum could be obtained by examining the negative logarithm of the intensities after subtracting a reference reading. Next, we bubbled pure nitrogen through the same solution for a further 5-10 minutes and obtained a spectrum, which reflected absorption by pure deoxy-hemoglobin. Using these two as approximate end points, it was then possible to produce a composite absorption curve for any value of blood oxygenation, and this

data could then be used to normalize tissue spectra after calculation of a relative value for oxygen concentration.

These two experiments have allowed us to account for, and hence normalize, different levels of saturation and amounts of blood on or within the tissue that is being sampled. This concept has also made us wonder if this technique could be used as a way of monitoring the level of oxygen saturation of blood at a single point and therefore as a monitor of certain treatments such as photodynamic therapy (PDT). This awaits further exploration.

Similar experiments to determine the absorption spectra, in the particular backscattering geometry of the instrument, have been conducted for the blue dye used in sentinel node location and are planned to determine the exact absorption pattern of β-carotene. The experiments to determine the absorption spectrum of blue dye involved diluting the dye in sterile water to obtain a series of solutions with varying concentrations of dye. The dye solutions were injected into a homogeneous fatty tissue phantom, for which pork fat and lard were used and readings were taken from the regions thus injected and from the unadulterated phantom. Comparing the spectra taken at different concentrations with each other and the background spectrum of the fatty matrix, normalizing these absorption coefficients and taking a weighted average gave a good approximation for the absorption spectrum.

Fiber probe development:

All fiber probes used in these studies have been designed and fabricated by co-investigators at Los Alamos National Laboratory and supplied to the clinical sites. The optical geometry of all the probes has been standardized so as to provide maximum consistency of measurement conditions for all studies. For all probe designs the illumination fiber (carrying light from the pulsed arc lamp to the tissue) has a core of 400 μm diameter and the signal (collection) fiber has a core diameter of 200 μm. The separation of the two fibers centers is fixed at 350 μm, which has previously been shown to maximize the spectral sensitivity to scattering from cellular nuclei. From Monte-Carlo modeling studies the predicted volume of tissue that is probed optically is approximately 500 μm long, 300 μm wide and 300 μm deep, although from our experimental studies, there is some effect from tissue up to about 1mm from the probe tip.

Two different types of probes were fabricated for the first series of clinical measurements: one type for interstitial measurements through a transdermal needle, and the other for direct superficial measurement during open surgery. For the interstitial application two variations of probe have been fabricated. One provides the smallest possible total probe diameter, for use through 18-gauge needles. This type has a polyamide outer protective sheath with an external diameter of approximately 900 microns. However, all transdermal optical measurements are correlated with a core biopsy from the same site, so a second transdermal probe was designed to fit the inner diameter of the guide needle part of the standard core-biopsy needle (MANAM™

automatic cutting needle). This probe has a surgical stainless steel sheath with a diameter of about 1.78mm, which permits easy insertion through the core-biopsy needle.

The other general type of probe was designed for easier manipulation by surgeons during breast surgery. It has a 5-mm diameter outer sheath of surgical stainless steel, and the fibers are fixed in the center, the remaining volume being filled with optically opaque epoxy. This probe design is easier to handle for surgeons wearing gloves, and helps to reduce the intensity of ambient light that reaches the collection fiber.

We have conducted studies of robustness of our probes against various types of sterilization. Ethylene oxide can be used with any of them, but that method is expensive and must be scheduled one or two days in advance of the expected use. The probes can also be disinfected with standard immersion fluids (such as glutaraldehyde), but this requires keeping the SMA connector ends of the probes out of the fluid because of possible corrosion of the polished metal mating parts. Moreover, many hospitals are reducing the use of biostatic fluids both because of potential harm to workers and environment, and because of some questions about reliability of sterilization. Ideally, we wish to be able to sterilize the probes by autoclave, which is reliable, safe and inexpensive. To that end we have changed some of the medical-grade materials used, especially the bonding epoxies, and have successfully tested the various components of the probes in several autoclave cycles, although we are still short of developing a fully autoclavable probe.

We have begun experimental testing of fiber probes using illumination fibers with larger numerical aperture than that of standard quartz fibers, as large as 0.40. We expect that this will permit tighter fiber bends without light loss, when compared with the standard fiber numerical aperture of 0.22. This would provide advantages for use in tight working environments, where the fibers may undergo severe bending between the probe and the spectrometer unit. We are also looking at developing smaller and more portable spectrometer units, which require less floor space in theatre, are easier to take from one location to another and are faster to set up, therefore allowing more data to be collected.

Patient enrolment, optical biopsy and histological sampling:

Clinical measurements are being taken with both types of fiber developed at Los Alamos. The first studies were undertaken on excised human breast tissue within 1 hour of surgery as in this situation it is straightforward to be sure that optical measurements and tissue biopsies are taken from exactly the same site. These optical readings were obtained with the transmitting and detecting optical fibers held in the rigid 5 mm diameter probe. This was convenient for laboratory and open surgery studies but was too large for percutaneous measurements for which the smaller flexible probe was developed, small enough to pass through an 18-gauge biopsy needle. Following the initial studies on excised breast tissue, optical measurements have been

obtained in three situations. All patients were enrolled into the study after the details had been explained to them and they had given full written consent.

To test the viability of taking ex-vivo measurements we conducted an experiment to determine how the optical properties of tissue change after removal from the body i.e. changes due to cooling, dehydration, loss of circulating blood and possible de-oxygenation. If we could show that the optical readings from breast tissue were essentially similar both *in-* and *ex-vivo* then it would be possible to obtain readings from mastectomy specimens immediately after excision in the operating room. This would greatly increase the number of data sets we could obtain for training the system. One method of testing this was to mark a small area of breast tissue in a tumor bed following wide local excision of a breast cancer and obtain an optical reading *in-vivo*. This area would then be excised with a small cuff of surrounding tissue and serial measurements would be taken over time on the same point. Alternatively in order to define the point of observation uniquely and maintain the position of the probe a cannula from an appropriately gauged needle was sewn into position within the incompletely excised specimen and a spectrum was obtained. After the excision was complete the probe was re-inserted into the cannula and a timed series of spectra were obtained over a period of up to two hours. Although variants of this procedure have been performed on five patients to date the numbers are too small to draw absolute conclusions although they strongly suggest that spectra obtained within a period of thirty minutes following excision contain very similar information to those taken in-vivo. Over time, a gradual shift in the oxygen absorption curves was seen, although the overall shape of the spectra did not change remarkably. Analysis is ongoing to determine if the small change is significant before embarking on obtaining further *ex-vivo* measurements. In the meantime we have concentrated on obtaining as many *in-vivo* measurements as possible by the methods outlined below with any ex-vivo measurements clearly labeled with the time elapsed after excision.

As each patient is enrolled in the study it is necessary to record certain relevant information in order to compare the measurements taken. This includes aspects of their medical history, including method and result of diagnosis, endocrine status menopausal status and details of the procedure being performed. In order to take the appropriate information from each patient we have developed a pro-forma, which can be completed from details in the patients' notes and then later added to an electronic database. An example of this pro-forma used is included as appendix two.

a) Percutaneous measurement:

These were done on patients undergoing surgery for either benign or malignant conditions of the breast. Once the patient was anesthetized, a core-cut (14 French gauge) biopsy needle was inserted into the tumor through the skin (in the case of cancers or suspected cancers, the needle was inserted through skin that would be removed as part of the surgical procedure). When it was in the correct position, the inner needle was removed and the thin optical probe inserted for measurements to be taken. Once a satisfactory spectrum had been obtained, the core-cut needle was

reassembled taking great care not to move the tip of the needle relative to the tumor. A standard tissue biopsy could then be obtained which contained the area of tissue that had been interrogated optically. After this, the scheduled surgery was performed.

In order to unambiguously correlate the histology with the spectra acquired it was necessary to indicate to the pathologists the portion of the acquired core that should be examined. The core-cut device takes a sample of tissue approximately 1.5mm in diameter and approximately 1cm long. Because the optical probe would only have measured the nearest regions of the core we adopted a system of marking the opposite end of the core, furthest away from the probe with India ink and then requesting that the histologist report the non-inked end of the sample separately. It was also agreed that the histologist would report the pathology of the sample according to certain specific criteria, which are detailed in a core cut biopsy reporting pro-forma. An example form is attached as appendix three and details the categories of normal tissue and benign or malignant breast pathology that the pathologist will classify the relevant end of the core into. Note that the statement requires the pathology within the first 2mm to be stated in accordance with the experimental work detailed above and in our previous report, as well as the pathology of the remainder of the core if different. This allows the technique to be assessed as a predictor of the pathology of the core and maybe as a guide to taking more accurate core-cut biopsies.

b) Examination of tumor bed:

Current trends in breast cancer care tend towards more conservative surgery where less breast tissue is removed. This is marked by the decrease in the number of mastectomies performed (removal of the whole breast) and an increase in the number of wide local excisions where the tumor is removed with between 1 and 2cm of normal breast tissue as a margin on all sides. During such wide local excision of a breast cancer, it is often difficult for the surgeon to identify the tumor margins just by direct inspection of the surgical wound. In this situation, biopsies are taken to see if there is still cancer present. These can be taken in the form of random biopsies from the quadrants of the cavity left after excision of the tumor, or as a set of cavity shavings where strips of tissue are removed from the walls of the cavity and sent separately for examination. When this was required, optical measurements were taken of the same site immediately prior to the conventional biopsy and the results correlated.

c) Sentinel and other axillary lymph nodes:

All breast cancers have the ability to metastasize to lymph nodes (glands) in the axilla. Knowledge of whether or not this has occurred is important in determining the stage of the cancer i.e. degree of spread. This gives an indication of a patient's prognosis and may determine if adjuvant treatment such as chemotherapy is warranted. The sentinel node is the first lymph node that drains the region of the breast containing the tumor and is now recognized as a marker for axillary lymph node involvement with cancer. If the sentinel node is clear of cancer then the chance of any other node being involved with cancer is extremely small [1].

The sentinel node can be identified by various dye labeling techniques including blue dyes that can be observed during surgery and colloids containing radioactive tracers that can be followed with a gamma ray probe. The optical absorption of the dyes must be taken into account when examining the spectrum of the lymph nodes. Once found and removed the sentinel node must be assessed histologically either by frozen section while the patient remains anesthetized, or later, by paraffin embedded section. If the node is involved with cancer, then the other nodes in the axilla need to be removed. It would be simpler if the other nodes could be removed at the same operation, but this is only possible if one can get a rapid answer on whether or not the sentinel node is involved. The optical biopsy could provide this answer. We are now taking optical measurements on sentinel nodes as well as other resected nodes, immediately after surgical excision and correlating the results with subsequent conventional histology. Further details of the methods are given in previous reports and published papers. The protocol for examining the nodes once excised was to bivalve the node, by cutting along the longest axis without completely separating the two halves. In the case of the nodes identified as sentinel impressions of the node were taken for imprint cytology by pressing the bivalved node several times onto a glass slide. This slide would be dried, sent to the lab and examined by a cytologist. Although this procedure was being performed as part of a separate study it enabled the measurements taken on the node to be compared to an additional conventional diagnosis technique. Optical measurements would be taken typically from four points on the bivalved surface of the node, typically representing the center of the node, or medullar and the periphery of the node, or cortex. This would be done on both bivalved surfaces, typically with two spectra per point giving a total of eight spectra and four points per node examined.

Data collection, recording and analysis:

All optical data collected from patients was automatically recorded on a laptop computer, which runs the optical biopsy system. This is backed up onto disc after each new data set. The raw data is transferred into a spreadsheet file along with the corresponding histological diagnosis. These files are used for analysis in both Los Alamos and London. Examples of the spectra obtained have been given in previous reports. In addition to the automatically recorded details, a paper pro-forma listing the position and clinical diagnosis of the spectra acquired was taken at the time of the procedure and later transferred into an electronic database. This allowed not only the histological (definitive) diagnosis but also the clinical suspicion to be compared to the recorded spectrum.

Development of spectral classification methods:

Initially two different automated methods of spectral classification were used to assess the degree of correlation between pathology and spectral pattern differences: artificial neural networks and hierarchical cluster analysis. Artificial neural networks (ANNs) were selected for study because of the expectation by our group and other researchers [2, 3] that ANNs would prove to be a generally useful method of tissue spectral

classification. ANNs are well suited for classification in systems where model-based classification is difficult. Such is the case with ESS spectra of breast tissue because of its remarkable heterogeneity of tissue types (comprising glandular, adipose, fibrous, tubular, connective and other tissue types) with consequent broad variability in optical scattering and absorption properties. Hierarchical cluster analysis (HCA) was selected for study as an alternative to the many approaches to classification that provide unbounded class regions (including linear discriminant analysis, regression analysis and ANNs). Further details of these approaches are given in our published papers and previous reports.

In the second year of the program while the analysis detailed above continued at Los Alamos, the analytical approach was expanded in London to concentrate on a model based analysis (MBA) approach where certain consistent features within the spectra are analyzed and accounted for. Such features include the hemoglobin concentration (as mentioned above in the section on experimental work), the presence of blue dye or an absorption spectrum for ß-carotene, which is only present in fat and not in tumors. In addition a number of invariant linear portions were noted by careful observation and linear approximations could be found for these sections. The remaining part of the procedure is to break the spectrum up into linear portions and using a process of line-fitting and repeated re-normalization reduce the raw input to a series of gradients, intercepts and absorption co-efficients. When referenced to experimental measurements the absorption coefficients can be successfully converted to concentrations (or saturations in the case of Hemoglobin). The reduced set of descriptors (~30 as opposed to 1800 values for the whole raw spectrum) could then be fed as inputs into an appropriate neural net or hierarchical cluster model, for convenience, and to avoid repetition of effort between London and Los Alamos, the analysis of the model based results was done with a linear discriminant based method using the package SYSTAT. This pre-processing could result in a dramatic reduction in overall processing time and also the ability to get relative values between different spectra at certain fixed points.

In the third year of the program continued work on the MBA approach led to a patent application for the spectral decomposition algorithms, which was submitted in February 2001 in the United Kingdom and subsequently in the United States. A completed form DD 882 in respect of this invention was submitted on December 15, 2000. Investigations are currently underway as to appropriate ways to commercialize the technology and the advances made as a result of this program.

Data analysis results:

Data sets are now available from a total of 154 patients recruited in London; this includes 17 patients recruited in a preliminary study. Data from these patients yielded a total of 647 matched pairs of optical and conventional histopathalogical data. Although on average of 2 or 3 spectra were taken from each tru-cut or tumor bed site there is an excellent correlation between successive spectra taken from the same site, shown statistically by r^2 values for the cross correlation between separate spectra of >95%

(>99% in the majority of cases). This shows consistency in the readings and allows multiple spectra from a single site to be averaged, further reducing the noise level. In the case of spectra acquired from different points on nodes the correlation is not so close so data acquired at different points had to be counted separately but with the same histological diagnosis. The sites studied included 100 percutaneous biopsies (TRU-CUT), 147 tumor margins examined during surgery, 127 specimens of breast tissue examined immediately after surgical removal and 264 from axillary nodes including 56 from nodes identified as sentinel nodes, also examined immediately after excision. Some measurements have been taken on exterior tissue to look at the possibility of identifying Paget's disease of the nipple in-situ. Currently this part of the investigation is at an early stage and although the diseases are related the spectra are not suitable for inclusion in the breast cancer analysis.

Because of the serious and prolonged technical problems encountered with the optical biopsy hardware and data collection at Little Rock it has been decided not to attempt to use any of the data collected there.

In total we have over 2700 reliable spectra from the 647 sites mentioned above. The conventional histology showed that the breast tissues examined included evidence of 79 invasive cancers, 9 showing carcinoma in situ, a couple only showing predominantly atypical ductal or lobular hyperplasia and 34 with benign breast disease (including fibrosis, sclerosing adenosis and other non malignant lumps). The rest showed a mixture of normal fibrous, fatty or lobular breast tissue. In the lymph nodes, 56 datasets showed metastatic cancer including two that showed micro-metastases, 69 displayed reactive changes while the remainder were examples of normal lymphatic tissue.

For all analyses the spectra from breast tissues and sentinel nodes were examined separately since they are basically different tissues. For the modes of analysis used at Los Alamos the spectra were pre-processed by first normalizing each spectrum to the same total integral over the spectral range of 350 to 750 nm ensuring that only spectral shapes were compared and not total scattering efficiencies. For the London analysis the pre-processing routines were more complex and some aspects have been described above.

In presenting the statistical results, sensitivity and specificity are defined in the standard way:

$$\text{Sensitivity} = TP/(TP + FN) \qquad \& \qquad \text{Specificity} = TN/(TN + FP)$$

Where TP, FP, TN and FN represent the numbers of true positives, false positives, true negatives and false negatives, respectively, as determined by the corresponding histopathology. For the training and testing of the ANN's we randomly chose 80% of the data samples as a training set, reserving the remaining 20% as the test set. This was repeated three times with three different random choices of the 80/20 split. Results

from the three different types of analysis for detecting cancer in both breast tissue and lymph nodes are shown below:

Table 1: Sensitivity and specificity for detection of cancer in breast tissue

	ANN	HCA	MBA
Sensitivity	69%	67%	94%
Specificity	85%	79%	92%

Table 2: Sensitivity and specificity for detection of cancer in axillary lymph nodes

	ANN	HCA	MBA
Sensitivity	58%	91%	84%
Specificity	93%	76%	87%

ANN: Artificial Neural Network, HCA: Hierarchical Cluster Analysis and MBA: Model Based Analysis

Complementary studies of scattering from cell suspensions:

Under independent funding from a NCI/NIH grant, Dr. Judith R. Mourant, a colleague of Dr. Bigio at Los Alamos, has been studying the intrinsic scattering properties of parallel cell lines (cancerous and non cancerous). These studies demonstrate that the optical geometry of the fiber probes, as utilized for our breast cancer study, accentuates sensitivity of the collected scattering spectrum to variations in nuclear size and density, which are associated with malignancy [4, 5].

Complementary studies providing further optical biopsy data:

Under separate hospital funding in London, Dr Laurence Lovat, a colleague of Prof Bown, has used the flexible optical probe to make optical measurements endoscopically on a range of lesions in the gastrointestinal tract that required a conventional biopsy, so the optical and conventional biopsies could be correlated. This study is showing particular promise in identifying areas of pre-malignant change in Barrett's esophagus, which cannot be seen by conventional endoscopy alone. This is an increasingly important problem as the incidence of cancers in this site is increasing faster than for any other solid organ. To date a total of over 800 measurements have been obtained from a group of over 200 patients and the initial results are looking extremely promising. The preliminary results identifying dysplasia within areas of Barrett's esophagus are given in table 3.

Table 3: Sensitivity and specificity for detection of pre-cancerous changes in Barrett's esophagus

		Elastic Scattering Spectroscopy	
		Dysplasia	No Dysplasia
Histology	Dysplasia	16	2
	No Dysplasia	10	50

Sensitivity	89%	Specificity	83%

A second study, in conjunction with the department of dermatology at UCL, has also been started where optical measurements are taken from suspicious pigmented skin lesions, 81 so far, which are undergoing surgical excision for histological analysis. The aim of this study is to develop an algorithm that will allow discrimination between benign and dysplastic naevi and malignant melanoma. This would have huge potential as a method of gaining immediate diagnostic information of any suspicious lesion so reducing the need for surgical biopsy. Early results have been encouraging and the increased numbers of matched pairs of optical and histology data obtained by these means have helped to develop techniques for analyzing the optical data from the breast studies.

Comment:

The results on breast tissue so far continue to be very encouraging and we hope that as the number of measurements increases, we will be able to consolidate and confirm the promising sensitivity and specificity found so far. Given the numbers of patients recruited in the last year it should be relatively straightforward to meet the new target of patient recruitment as set out in the revised statement of work. A lot more work is required to find the best way to analyze the spectra we have. We need to identify the features that best distinguish between normal and malignant tissue and to find as simple a way as possible to interpret the results to minimize the computing time required. The model-based analysis developed over the last year seems a lot simpler than the artificial neural networks and hierarchical cluster analysis used in the first year and is giving comparable results, so this will be explored further in the next year. The hope is that an algorithm can be developed for rapid analysis of spectra so that in the clinical setting, the technique can be used to give almost immediate discrimination between benign and malignant tissue with a reliability that is comparable to conventional histology.

Objective 2: Improve treatment of breast cancer by using ILP.

2A: Detection of residual cancer after ILP by contrast enhanced MRI:
2B: Real-time, dynamic monitoring of ILP in an interventional MR scanner:

Pilot studies for these 2 objectives were undertaken prior to commencement of the current program, although since then various practical and administrative problems have been encountered, which have seriously delayed patient recruitment.

Patient selection:

The patients proposed for this study were those with a proven small (up to 2cm) carcinoma in the breast who were scheduled for routine surgery (wide local excision or mastectomy). Further exclusion criteria were no previous radiotherapy or surgery to the lesion to be treated and no contraindication to MR imaging agents.

Needle insertion and imaging during laser therapy:

The systems planned were different in the two centers. In London, the preliminary real time studies were done in a 1T MR scanner (Siemens). This gave good real time images of the developing area of laser induced necrosis, but the configuration of the scanner made it difficult to keep the needles and laser fibers in the correct position as they had to be inserted with the patient outside the scanner and tended to be dislodged sliding the patient into the magnetic field. To overcome this problem, a 0.2T interventional MR scanner was purchased. It was thought that this field would be strong enough to get real time images of the thermal effects produced by the laser, but at the time no data were available to see if this was true. Unfortunately, the 0.2T field has proved to be too weak to get reliable real time images of the laser effects. We have tried to find new pulse sequences to overcome this problem, but so far, have not succeeded. Thus, it has not been possible to get new data on real time imaging of ILP in London. A new coil for use with the 1T scanner was purchased (with separate funding), which allows easier access to the breast. It was hoped that this would allow real time imaging to recommence, but unfortunately this has not proved practical either.

The protocol was to undertake contrast enhanced MR scans to establish the full extent of the cancer being treated. This was to be done with Fast Low Angle Shot (FLASH) sequences in London and with RODEO sequences in Little Rock with needle insertion either under ultrasound scanning (if the lesion was easily visible with this method) or MR guidance in the interventional scanner. This was to be followed by ILP treatment, aiming to completely ablate the small cancers, assess the completeness of ablation on contrast enhanced MR scans shortly after ILP (up to a few days) prior to surgery and then carefully examine the surgical specimen to see if the MR scans detected any untreated areas of cancer.

Results:

So far, we have failed to get any new usable data in this part of the program. In London, several other research programs on early breast cancer started at the same time as this one resulting in competition for recruitment of suitable patients. We have tried to tackle this by making arrangements for appropriate patients to be identified in other hospitals and then referred to us, but we still do not have any usable data. Progress has been further handicapped by the emigration of the senior surgeon in this program and the retirement of the other senior breast surgeon in the hospital together with many months of inability to undertake interventional procedures by the interventional radiologist following a skiing accident. The situation is now better as 2 new breast surgeons have been appointed and are in post and we hope to recruit at least some patients to this part of the study over the next year.

Four suitable patients were identified in Little Rock, who received laser treatment, but documentation to correlate before and after treatment scans with histology was incomplete. The program in Little Rock has now ceased.

2C: Long term healing after ILP to benign fibroadenomas of the breast.

This study was undertaken to understand how laser treated areas in the breast heal as it was felt inappropriate to leave laser treated cancers in the breast for an extended period of time at an early stage of this project. The technique of treatment was essentially the same as for interstitial laser photocoagulation of breast cancers, but most of the imaging was done using ultrasound rather than MRI. This was done for several reasons: fibroadenomas are much easier to define on ultrasound than MRI, so the simpler and cheaper imaging option was chosen and further, if part of the lesion was missed or inadequately treated, it would not have the same serious consequences for the patient as if part of a cancer was missed.

The patients included in this study were those with palpable breast lumps confirmed to be benign fibroadenomas on clinical examination, ultrasound scan and fine needle aspiration. Some of the patients included in this study were first treated prior to the start of the present grant but their follow up was completed as part of the current program. Technical details are given in the publication "Interstitial Laser Photocoagulation for Fibroadenomas of the Breast" which was appended to an earlier report. The key early findings (on the first 12 lesions scanned a year after treatment) were that the median reduction in lesion size after treatment, as measured on ultrasound, was 38% at 3 months, 60% at 6 months and 100% at 12 months. None of the lesions examined at one year could be detected clinically and only one was detected on ultrasound. The treatment was a simple day case procedure. The only complication of note was a minor skin burn seen in 3 patients early in the series and since the technique was modified to protect the skin by cooling, this has not occurred again.

In addition to the patients mentioned above another 30 fibroadenomas have been treated in 26 patients recruited since the start of the program, and twelve month follow up is now available on nine of these patients. A control group of 15 patients with a total of 27 fibroadenomas has also been identified to monitor for the natural history of the condition and statistically significant differences have been observed between the treated and untreated groups that will be reported shortly. Full follow up results on as many of these individuals as possible should be available at the end of this program in another year's time. In addition the revised statement of work commits us to recruiting a further 10 patients into the study over the next twelve months, although the full period of follow up will not be available for these patients.

One of the anxieties at the outset of this program for treating breast cancer was that laser treatment, even if completely successful, would replace a palpable cancer in the breast with a palpable lump of scar tissue. We thought this would cause patients considerable anxiety, as they would worry that the cancer was still there. We have been very encouraged to find from this study on fibroadenomas that all the tissue necrosed by laser treatment appears to be resorbed without leaving any palpable scar tissue, although this can take up to a year. This will make ILP a more attractive option to many patients.

2D: Delayed surgery after ILP in patients over 65 years treated with Tamoxifen.

This study was undertaken as a first step to understanding how laser treated breast cancers heal if they are not removed by conventional surgery shortly after ILP. This group of patients was chosen, as treating them with tamoxifen to shrink the cancer for 3 months prior to surgery is an accepted treatment option in the United Kingdom. The technique for the laser treatment itself is exactly the same as for patients treated with ILP just before surgery. Treatment was planned to cover the entire tumor, but the main aim of the study was to see if MRI could detect incompletely ablated cancers, so it was desirable that in some cases, ablation should be incomplete. This was acceptable as all patients were being treated with tamoxifen. All patients have pre-ILP, interim (6 weeks) and pre-surgery (3 months) MR scans (contrast enhanced) and the scans are correlated with the histological findings on the surgical specimen.

Results:

The number of patients suitable for this study is quite small and it was only ever planned that this should be carried out in London. To date, 8 patients have been recruited for ILP and so far 4 have had surgery 3 months later. In the surgical specimens, 2 patients had residual tumor. In one patient this was detected as a 2mm area of increased enhancement on the pre-surgery scan. As the specimens are orientated in the transverse plane, we were able to confirm that the abnormality on the MR scan corresponded exactly with the abnormality seen on histology. In the second patient, there was minimal enhancement on pre-operative scans so attempts to identify the extent of residual tumor on MR after treatment were unsatisfactory.

Comment:

The number of patients recruited so far on this protocol is small, but it is encouraging that a residual cancer as small as 2mm could be detected and that no areas of viable cancer were found on the surgical specimens that had not been seen on the MR scans. Recruitment for this study will continue over the next year.

7. KEY RESEARCH ACCOMPLISHMENTS

Part 1: Optical diagnosis of breast cancer:

Reliable optical probes have been developed which are suitable for examination of tissue exposed at open surgery, ex-vivo tissue (lymph nodes) and for percutaneous use through needles.

Optical spectra from tissue are straightforward to take and are reproducible, although there are still technical problems with some of the recording equipment.

Spectral data sets have been analyzed using Artificial Neural Networks (ANN), Hierarchical Cluster Analysis (HCA) and the much simpler model based analysis (MBA). The MBA undertaken recently has given results comparable to those from ANN's and HCA's but needs much less computer power, so may be a better way forward.

The latest results using model based analysis for detection of cancer in breast tissue gave a sensitivity of 94% and a specificity of 92% and for detection of metastases in axillary lymph nodes the corresponding sensitivity and specificity values were 84% and 87%.

Further work is required to improve the data analysis and to increase the size of the data sets, but these early results are most encouraging and suggest that the technique of optical biopsy might develop a useful role in clinical practice.

2. Optical treatment of breast cancer: Interstitial Laser Photocoagulation (ILP):

Complete ablation of small, localized breast cancers with ILP is possible.

Residual tumor left after ILP as small as 2mm in diameter can be detected on contrast enhanced MR.

It is technically difficult to position the laser fibers at the correct sites, but systems are being developed to do this more easily and accurately.

Laser induced thermal destruction of cancers can be monitored by real time MR scanning, but it requires a high magnetic field (1T is adequate but 0.2T is not). It is unlikely to be possible to proceed further with real time monitoring for destroying small breast cancers until open, interventional MR scanners with a higher magnetic field are available or better ways are devised for manipulating needles in the breast within a closed scanner.

It may soon be possible to start studies treating small breast cancers with ILP without subsequent surgical excision, but it is necessary to get more data on MR imaging of small, laser treated cancers, which are subsequently excised, to be sure that any inadequately treated areas of cancer can be detected.

MRI guided ILP may have lower costs and provide better cosmesis than surgical lumpectomy.

Long-term follow up of fibroadenomas treated with ILP shows that the necrosed tissue is safely and completely resorbed without leaving a lump. It was not an original aim of the program, but this part of the study has shown that ILP is a simple and effective alternative to surgery for fibroadenomas. This could become a particularly

attractive option in routine practice, as the condition is common in young women who are naturally keen to avoid having a scar on their breast.

8. REPORTABLE OUTCOMES

Journal articles / Book chapters:

1. Harms SE, Mumtaz H, Hyslop B, Klimberg S, Westbrook K, Korourian S: Magnetic Resonance Imaging Directed-Laser Lumpectomy. *Breast Diseases, A Year Book Quarterly,* St. Louis; Mosby. 1998.

2. LM Lai, MA Hall-Craggs, H Mumtaz, PM Ripley, TI Davidson, MW Kissin, C Saunders, I Taylor, SG Bown. Interstitial laser photocoagulation for fibroadenomas of the breast. The Breast 1999; 8:89-94.

3. Harms SE, Mumtaz H, Hyslop B, Klimberg S, Westbrook K, Korourian S: RODEO-MRI guided laser ablation of breast cancer. SPIE 1999; 3590:484-489.

4. C. D. O. Pickard, G. M. Briggs, L. Lovat, C. Saunders, P. M. Ripley, I. J. Bigio and S. G. Bown. Elastic Scattering Spectroscopy In-Vivo: Optical Biopsies of Cancers of the Breast and GI Tract. SPIE Proceedings Vol.3917

5. C. D. O. Pickard, G. M. Briggs, C. Saunders, P. M. Ripley, I. J. Bigio and S. G. Bown. Optical Biopsy: In-Vivo diagnosis of Breast Cancer using Elastic Scatter Spectroscopy. SPIE Proceedings Vol.3907, pp.592-599.

6. P. M. Ripley, D. Pickard, I. G. Rose, C. A. Kelley, I. J Bigio, G. Briggs, L. Lovat and S. G Bown. A Comparison of Artificial Intelligence Techniques for Spectral Classification in the Diagnosis of Human Pathologies based upon Optical Biopsy. OSA Biomedical Topical Meetings Proceedings, 2000, MC5

7. Bigio IJ, Bown SG, Briggs GM, Lakhani SR, Pickard CD, Ripley PM, Rose IG, Saunders CM: Diagnosis of breast cancer using elastic-scattering spectroscopy: preliminary clinical results. Journal of Biomedical Optics 2000; 5(2):221-228

8. Hall-Craggs MA, Smart S, Gillams A, Lees WR. MR monitored minimally invasive thermal therapies to the body. Editor I Young. John Wiley and Sons Ltd.

9. Hall-Craggs MA. Interstitial ablation therapy to breast tumors. European Journal of Radiology 2000;10(1): 59-62

10. AC Lee, CDO Pickard, MRS Keshtgar, SG Bown, GM Briggs, S Lakhani, IJ Bigio, PJ Ell. Intra-operative assessment by optical biopsy for sentinel lymph node metastasis in breast cancer. British Journal of Cancer 2001; 85: Supplement 1 p. 27 **Included as Appendix 4**

Presentations:

The Wendy & Emery Reves Internationl Breast Cancer Symposium October 1998, Dallas.
"RODEO MRI Guided Laser Lumpectomy: The potential for treatment without disfigurement". Harms S.

Radiology Society of North America (RSNA). Nov 1998, Chicago.
"Laser Lumpectomy with interactive MR imaging: Histopathological correlation". Harms S.

The International Society for Optical Engineering (SPIE). Jan 1999. San Diego.
"RODEO MRI guided laser ablation of breast cancer". Harms S.

16th Annual Miami Breast Cancer Conference. Feb 1999. Miami.
"Integration of MRI and treatment planning". Harms S.

British Breast Group. May 1999 Edinburgh.
"Shedding Light on Breast Cancer". Saunders CM.

Biomedical Optical Spectroscopy and Diagnosis, (Munich, June 1999), OSA TOPS Vol. (1999, in press).
Invited: "Optical Diagnosis and Treatment of Breast Cancer," Bigio IJ. and Bown SG.

Interventional MRI , British Institute of Radiology. June 1999, London.
"Thermal Ablation of Breast Tumors". Briggs GM.

Light for Life, (Cancun, Mexico, July 1999), Springer Verlag (1999, in press).
Invited Plenary: "Minimally-invasive optical diagnosis and treatment of breast cancer," Bigio IJ. and Bown SG.

Advances in Optics for Biotechnology, Medicine and Surgery, 1-6th August 1999, Kona, Hawaii, USA.
"Diagnosis and Treatment of Breast Tumors through the combined use of Optical Biopsy and Interstitial Laser Photocoagulation". Ripley PM.

6th Nottingham International Breast Cancer Conference. Sept 1999, Nottingham.
"Lasers – A minimally invasive way forward for breast tumors". Briggs GM

Magnetic Resonance Radiologists Association. Oct 1999. London.
"Breast Biopsy and interstitial therapies". Hall-Craggs MA.

Breakthrough Breast Cancer meeting. Nov 1999, London.
"New methods of breast biopsy and minimal invasive techniques". Hall-Craggs M.
"Optical diagnosis of breast cancer". Bown SG.
"Local destruction of breast cancer with laser". Bown SG.

High Care 2000, Feb 2000, Bochum, Germany
"Optical Biopsy for Minimally-Invasive Diagnosis of Breast Cancer" Irving J. Bigio, Stephen G. Bown, Gavin Briggs, Christine Kelley, Sunil Lakhani, David Pickard, Paul M. Ripley, Ian G. Rose and Christobel Saunders

Era of Hope. June 2000, Atlanta.
"Interstitial laser photocoagulation for treatment of breast tumours". Oral presentation by Bown, S.G. and poster presentation by Briggs, G.M.
"Optical biopsy for the diagnosis of breast tumours". Pickard C.D.O.

British Society for Surgical Oncology, Nov. 2000, Nottingham
"Histological Assesment of Breast Tissue by Optical Biopsy" Briggs, G. M.
"Optical Biopsy for the Assessment of Sentinel Lymph Nodes of the Breast" Briggs, G. M.

Second International Congress on MR Mammography, Sept 2000, Jena Germany
"Laser Treatment in Benign Breast Disease" Hall-Craggs, M. A.
"RODEO MRI Directed Laser Lumpectomy" Harms, S.
"Minimally Invasive Therapy in Breast Cancer" Hall Craggs, M. A.
"Optical Biopsy of Breast Tissues" Bigio, I. J., Bown, S. G., Briggs, G. and Hall-Craggs, M. A.

European Conferences on Biomedical Optics, Munich, June 2001
"Optical biopsy for the diagnosis of breast tumours."
CDO Pickard, S Lakhani, IJ Bigio, SG Bown, GM Briggs, AC Lee, PM Ripley
"Interstitial Laser Photocoagulation of Breast Tumours"

1st Int. Conf. on Sentinel Node Biopsy in Mucosal Head and Neck Cancer, Jun 2001, Glasgow
"Optical Biopsy for the Assessment of Sentinel Lymph Nodes". Pickard et al.

Informatics:

Set-up of a computer database, (Microsoft Access) for patient records and a second for correlation of spectral data and histological findings. This includes patient details, treatment parameters, radiological and clinical follow-up, histology and any complications.

9. CONCLUSIONS

The work on optical diagnosis of breast cancer has proceeded extremely well. We now have a reliable system for taking optical readings at open surgery and through a biopsy needle. Preliminary analysis of the first sets of paired optical and histological data by artificial intelligence techniques has shown reasonably high specificity and sensitivity for the detection of cancer in breast tissue and sentinel nodes. The work in the second year using model based analysis has suggested that this simpler method may reduce the computing power required without jeopardizing the quality of the results, and further work in the 3rd year to refine this approach has resulted in a patent application. These good early results are encouraging, but much more data are required to know how reliable these measurements are. It is hoped that enough further data can be gathered over the next year to prepare a relatively simple algorithm for spectral analysis that can be run on a laptop or palmtop computer to give an immediate indication of whether a particular spectrum is from normal or malignant tissue. If successful, this technique may be able to provide immediate pathological information that is comparable to that provided by conventional histology.

The studies on laser treatment of cancers have been seriously limited due to technical problems and difficulty recruiting patients. Nevertheless, it does seem feasible to completely ablate small cancers. Areas of viable cancer as small as 2mm in diameter remaining after ILP can be detected on contrast enhanced MR. We have shown that laser induced thermal changes can be detected in real time during laser energy delivery and that these may be able to predict when enough heat has been delivered to destroy the entire cancer. However, it has also become clear that there are considerable technical problems involved in optimizing all aspects of the treatment. Real time monitoring only works in high magnetic field scanners (1.5T), but easy insertion of the fibers requires an interventional scanner, most of which currently only operate at low magnetic fields. This program has the potential to provide a safe, relatively straightforward and effective treatment for selected small breast cancers that may have lower costs and better cosmesis than surgical lumpectomy.

The long-term results from treating benign fibroadenomas have taught us a lot. The necrosed tissue is resorbed completely without leaving a residual lump of scar tissue in the breast and without any long-term complications. This is very reassuring, as necrosed cancer tissue is likely to be resorbed in the same way. It is also good for patients psychologically as if any women could feel a residual lump in the breast after ILP for cancer, she would worry that there was still some malignant tissue left behind, even if repeat biopsies showed no evidence of cancer. This particular study was undertaken to understand how ILP treated cancers could be expected to heal. However, the results so far have been so impressive that if they are confirmed in the larger number of similar patients being treated in the present study, we are considering offering ILP as a routine treatment for benign fibroadenomas in patients who want active treatment but do not want open surgical excision. This could be of particular importance to women who have multiple fibroadenomas or who have a tendency to

keloid formation as the cosmetic result would be so much better than conventional surgery.

With the acceptance of the revised statement of work (March 2001) studies on laser treatment of cancers will continue in the next year. However, the withdrawal of Little Rock from the project and the fact that the existing problems of recruiting suitable patients in London are likely to continue, will limit the amount of new information we can generate on this aspect of the program. Further data will be gathered on the long term follow up of ILP treated fibroadenomas. However, our most important advances have been made in the optical diagnosis part of this program and we are confident that we can continue to make rapid progress on this in the coming year.

References:

1. Snider, H., *et al.*, *Sentinel node biopsy in the staging of breast cancer.* American Journal of Surgery, 1998. **176**(4): p. 305-10.
2. Ge, Z., K.T. Schomaker, and N.S. Nishioka, *Identification of colonic dysplasia and neoplasia by diffuse reflectance spectroscopy and pattern recognition techniques.* Appl. Spectroscopy, 1998. **52**: p. 833-839.
3. Osbourn, G., *et al.*, *Automated pattern recognition based on the visual empircal region of influence (VERI) method: A users guide.* .
4. Mourant, J., *Evidence of intrinsic differences in the light scattering properties of tumorogenic and non-tumorogenic cells.* Cancer Cytopathology, 1998. **84**(366-374).
5. Mourant, J., *Mechanisms of light scattering from biological cells relevant to non-invasive optical tissue diagnostics.* Appl. Optics, 1998. **37**: p. 3586-93.
6. Harms, S.E., *et al.*, *MR imaging of the breast with rotating delivery of excitation off resonance: clinical experience with pathologic correlation.* Radiology, 1993. **187**(2): p. 493-501.
7. Harms, S.E., *et al.*, *MR imaging of the breast: current status and future potential.* AJR. American Journal of Roentgenology, 1994. **163**(5): p. 1039-47.
8. Cross, M.J., *et al.*, *New horizons in the diagnosis and treatment of breast cancer using magnetic resonance imaging.* American Journal of Surgery, 1993. **166**(6): p. 749-53; discussion 753-5.

Appendix 1

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
ANDREW C LEE	CLINICAL RESEARCH FELLOW

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
UNIVERSITY OF GLASGOW	MB BS	1995	Medicine
ROYAL COLLEGE OF SURGEONS OF EDINBURGH	FRCS	1998	Surgery

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. DO NOT EXCEED THREE PAGES.

PROFESSIONAL EXPERIENCE

Present Appointment (from 1st November 2000):
Clinical Research Fellow in the Treatment of Breast Cancer, National Medical Laser Centre, Department of Surgery, Royal Free and University College London Medical School

Previous Appointments:

Aug 2000 to Oct 2000	Senior House Officer, General Surgery (Gastrointestinal Unit), Gartnavel General Hospital, North Glasgow University Hospitals NHS Trust, Glasgow
Aug 1999 to Aug 2000	Senior House Officer, General Surgery (Breast & Surgical Oncology Unit), Western Infirmary, North Glasgow University Hospitals NHS Trust, Glasgow
Aug 1998 to Aug 1999	Senior House Officer III. General Surgery at Southern General Hospital, South Glasgow University Hospitals NHS Trust, Glasgow
Feb1998 to Aug 1998	Senior House Officer, Neurosurgery, The Royal London Hospital, London
Aug 1997 to Feb 1998	Senior House Officer, General Surgery, Newham General Hospital, London
Feb 1997 to Aug1997	Senior House Officer,Accident and Emergency, Newham General Hospital, London
Aug 1996 to Feb 1997	Senior House Officer, Orthopaedics and Trauma, Newham General Hospital, London
Feb 1996 to Aug1996	Junior House Officer, General Medicine, Southern General Hospital, Glasgow
Aug1995 to Feb1996	Junior House Officer, General Surgery, Southern General Hospital, Glasgow

Career Development

Since qualifying in Medicine in 1995, I have worked in general surgery and related specialties. I have just joined the National Medical Laser Centre, University College London Medical School as a Clinical Research Fellow. I intend to pursue a career in General Surgery with clinical and academic interest in breast surgery. Over the past two years, I have consolidated my clinical and operative skills. Prior to embarking upon higher surgical training, I intend to focus my training in full-time research aiming for a MD degree, and to seek greater opportunities to publish and to present my research. Hopefully, these will provide me a platform for further research in my future career.

Appendix 2

OPTICAL BIOPSY BREAST

Patient details

Name: Study no: L
Hosp no: Date:
DOB: Ethnicity:

Clinical details

Pre-op Dx: Side:

Main comp: Site:

Palpable: FNA:

Core: Mammo:

U/S: Other Ix:

Previous breast history

Prev Hx	Side	Site	Surg	Chemo	Radio	Tam/Ari

FHx:

Smoking: Alcohol:

Endocrine profile

Menarche: Menopause:

Pres cycle: Para:

Age 1st preg: Br feed:

OCP: ( ) HRT:

Operation details

1° procedure: Inc time:
Exc time:

Ax surg: Inc time:
Exc time:

Appendix 3

OPTICAL BIOPSY PROFORMA

Specimen details

Patient no: L Patient initials:

Histology no: Core number:

Assessment of pathology between 0 to 2mm from non-inked end of core:

(Please tick following appropriate pathology)

Parenchyma Fat

Benign lesions:

Fibroadenoma "Fibrocystic change"

Solitary papilloma Solitary cyst

Multiple papilloma Periductal mastitis / Duct ectasia

Complex sclerosing Sclerosing adenosis

Radial scar Others (please specify)_____

Epithelial proliferation
Not present Present with atypia (ductal)

Present without atypia Present with atypia (lobular)

Pre-malignant & malignant pathologies:

DCIS (Growth pattern.....) LCIS

Grade 1 2 3

Microinvasion

Ductal carcinoma Lobular carcinoma

Ductolobular carcinoma Others (please specify)_____

Tubule formation 1 2 3 Nuclear pleomorphism 1 2 3

Mitotic frequency 1 2 3 Grade I II III

Pathology beyond 2mm from non-inked end:

Please state:_____

Examiner initials: Pathologist initials:

Appendix 4
Abstract from British Journal of Cancer 2001; 85: Supplement 1 p. 27

INTRA-OPERATIVE ASSESSMENT BY OPTICAL BIOPSY FOR SENTINEL LYMPH NODE METASTASIS IN BREAST CANCER.

AC Lee*[1], CDO Pickard[1], MRS Keshtgar[2], SG Bown[1], GM Briggs[1], S Lakhani[3], IJ Bigio[4] and PJ Ell[5]
[1]National Medical Laser Centre, [2]Department of Surgery, [3]Department of Histopathology, [5]Institute of Nuclear Medicine, Royal Free and University College Medical School, University College London, Charles Bell House, 67-73 Riding House Street, London W1W 7EJ, [4]Department of Biomedical Engineering, University of Boston, USA

Aims: The histological status of the axillary lymph nodes remains one of the most important prognostic indicators in breast cancer patients. It was the aim of this study to evaluate the accuracy of optical biopsy[1] (OB) as an intra-operative diagnostic tool to determine the histological status of the sentinel lymph node (SLN) in patients with invasive breast cancer.

Procedures: Since October 1998, A total of 51 patients had been enrolled in the second phase of this study. The median age of the patients was 52 years (range, 34 to 88years). After harvesting, the SLN was bivalved. Optical spectra were acquired using a clean probe from a number of representative points on the cut surface. The SLN was sent for histopathology.

Results: A total of 77 SLN were biopsied from 51 patients (1.5 SLN per patient). The sensitivity of this technique was 87.1%; the specificity was 85.2%.

Significance: Current intra-operative methods of assessing SLN for metastasis in breast cancer are fresh frozen section and imprint cytology. These techniques are operator dependent and time consuming. OB has the potential to provide an instant, non-operator dependent assessment of sentinel nodes.

Conclusion: OB has the potential to provide instant and non-operator dependent intra-operative analysis of SLN in patients with breast cancer, which will enable the surgeon to decide on performing axillary lymph node dissection at the time of initial surgery. Sensitivity and specificity should increase as the database of correlated biopsies increase in size.

Reference:
1 Bigio IJ, Bown SG, Briggs G et al. 2000 J. Biomedical Optics. 5(2); 221